

1. A method for preparing a peptide ester, the method comprising:  
providing an acidified alcohol solution;  
providing a peptide sample comprising a peptide species; and  
mixing the acidified alcohol solution and the peptide sample to form a mixture

5 and thereby generate an ester of the peptide species, wherein concentration of the peptide species in the mixture is less than 1 nM.

2. The method of claim 1, wherein the alcohol is selected from the group consisting of methanol, ethanol, propanol, and isopropanol.

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3. The method of claim 1, wherein the alcohol is a substituted alcohol selected from the group consisting of aminoethanol, trialkyl ammonium ethanol, biotinylated alcohol, and histidine labeled alcohol.

15 4. The method of claim 2, further comprising adsorption of the peptide species onto a solid phase prior to the mixing step.

5. The method of claim 4, wherein the solid phase comprises a hydrophilic chromatography phase.

20 6. The method of claim 4, wherein the solid phase comprises a strong cation exchanger.

7. The method of claim 1, wherein the ester is a methyl ester.

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8. The method of claim 1, further comprising sequencing the peptide species after generation of the ester.

30 9. A method for preparing a peptide methyl ester, the method comprising:  
providing a first solution comprising diazomethane and a solvent, wherein the solvent is miscible in water; and

mixing the first solution with a second solution, wherein the second solution is aqueous and comprises a peptide species, to thereby form a methyl ester of the peptide species.

5        10. The method of claim 9, wherein the solvent is selected from the group consisting of methanol, ethanol, isopropanol, acetonitrile, ethanolamine and triethanolamine.

10      11. The method of claim 9, further comprising sequencing the peptide species after formation of the methyl ester.

15      12. A method of determining the relative quantity of a peptide species in a mixture of peptides, the method comprising:

      providing a first sample comprising a first population of the peptide species, wherein the concentration of the peptide species in the first sample is less than 1 nM;

      esterifying the first population of the peptide species to form a first population of peptide esters;

      providing a second sample comprising a second population of the peptide species, wherein the concentration of the peptide species in the second sample is less than 1 nM;

      esterifying the second population of the peptide species with an isotopically enriched reagent to form a second population of isotopically labeled peptide esters;

      mixing the first population of peptide esters with the second population of isotopically labeled peptide esters to form a mixture;

      separating the mixture into a plurality of fractions;

25      20     analyzing a fraction with a mass spectrometer to obtain a first signal for the first population of peptide esters and a second signal for the second population of isotopically labeled peptide esters; and

      determining the relative quantity of the peptide species in the first sample as compared to the second sample.

13. The method of claim 12, wherein the first population of peptide esters comprises peptide methyl esters.

14. The method of claim 12, wherein the second population of isotopically  
5 labeled peptide esters comprises peptide methyl esters.

15. The method of claim 12, wherein the second population of the peptide species is labeled with a stable isotope selected from the group consisting of deuterium, carbon-13, nitrogen-15 and oxygen-18.

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16. The method of claim 12, wherein the second population of the peptide species is esterified using a solution comprising an alcohol.

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17. The method of claim 12, wherein the second population of the peptide species is esterified using a solution comprising a substituted alcohol.

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18. The method of claim 12, wherein the first sample and second sample comprise biological material derived from the same cell type or tissue type.

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19. The method of claim 12, wherein the first sample and second sample comprise biological material derived from different cell types or tissue types.

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20. The method of claim 12, wherein the determining step comprises ascertaining the ratio of hydrogen in the first population of peptide esters to deuterium in the second population of isotopically labeled peptide esters.

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21. The method of claim 12, further comprising sequencing the peptide species after determining the relative quantity of the peptide species.

22. A method of fractionating peptides, comprising:  
providing a sample comprising a plurality of different peptides;

adsorbing the peptides onto a strong cation exchanger;  
selectively desorbing a first subset of the peptides by the action of a first mobile phase;  
5 adsorbing the first subset of peptides onto a reversed phase HPLC column;  
selectively eluting a second subset the peptides from the reversed phase HPLC column by a second mobile phase that develops an increasing acetonitrile concentration gradient; and  
collecting peptide fractions eluted from the reversed phase HPLC column.

10 23. The method of claim 22, wherein the reversed phase HPLC column is a micro-HPLC column comprising both a reversed phase and a strong cation exchanger stationary phase, and wherein the second mobile phase comprises both an acetonitrile gradient and a pH gradient.

15 24. The method of claim 23, further comprising sequencing a peptide contained in a peptide fraction by mass spectrometry.

20 25. A peptide separation system, comprising:  
a column comprising an ion exchange stationary phase and a reversed phase HPLC phase, wherein the ion exchange stationary phase has strong cation exchange characteristics, and wherein the reversed phase HPLC has C2, C4, C8, C18 or polymeric characteristics; and  
a mobile phase gradient comprising an acetonitrile gradient and a pH gradient.